IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Shaharyar Khan		
Serial No.:	10/561,829	Group Art Unit:	1633
Filed:	March 23, 2007	Examiner:	Qian Janice Li
For:	*MODIFIED VECTORS FOR ORGANELLE TRANSFECTION*		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.131

Sir:

I, Shaharyar Khan, hereby declare that:

1. I am the inventor of the claimed subject matter in the above-identified patent application.

2. Prior to March 31, 2003, I worked in the United States to conceive and reduce to practice: (1) a viral capsid fusion protein including a protein transduction domain and an organelle localization signal, and (2) a viral vector containing the viral capsid fusion protein.

3. I used standard polymerase chain reaction (PCR) techniques and restriction fragment cloning techniques to prepare expression plasmids encoding: (1) the phage capsid protein gpD (see Exhibit 1B); (2) a TAT protein transduction domain and the phage capsid protein gpD (see Exhibit 1A); and (3) a TAT protein transduction domain, the phage

capsid protein gpD, a mitochondrial localization signal, and green fluorescent protein (GFP) (Exhibit 1C).

4. The expression plasmids were transformed into bacteria. DNA was isolated from the transformed bacteria and used as templates for construct-specific PCR to screen for bacterial clones containing the construct.

5. Exhibit 1 is a page from my laboratory notebook. The date is redacted and the data is from experiments conducted prior to March 31, 2003. Exhibits 1A, 1B, and 1C are three agarose gels showing the results of construct-specific PCR of three different cDNA constructs. The arrow in Exhibit 1A identifies the band resulting from PCR amplification of a construct encoding a TAT protein transduction domain fused to the cDNA of the phage capsid protein gpD. The band was detected in lanes 2-3 and 5-7, indicating that the corresponding clones are positive for the expression plasmid containing the construct.

6. The arrow in Exhibit 1B shows the results of PCR amplification of a construct encoding the cDNA of gpD phage capsid protein. The band was detected in lanes 2-8, indicating that the corresponding clones are positive for the expression plasmid containing the construct.

7. The arrow in Exhibit 1C shows PCR application of a construct encoding a viral capsid fusion protein including a protein transduction domain and an organelle localization signal. The construct includes a TAT protein transduction domain, gpD phage capsid protein, the mitochondrial localization signal of subunit VIII of human cytochrome

c oxidase, and GFP. The band was detected in lanes 3-6, indicating that the corresponding clones are positive for the expression plasmid containing the construct.

8. Once positive clones were identified, the expression plasmid encoding the viral capsid fusion protein including a protein transduction domain and an organelle localization signal was expressed in BL21 competent cells. The fusion protein was purified using a 6XHis-tag which was incorporated into the construct during the cloning process. Once isolated, the viral capsid fusion protein was added to a viral packaging extract containing the necessary viral proteins to enable formation of active virions containing the viral capsid fusion protein.

9. As shown in paragraphs 3-8, I constructed and produced a viral vector containing a viral capsid fusion protein including a protein transduction domain and an organelle localization signal prior to March 31, 2003.

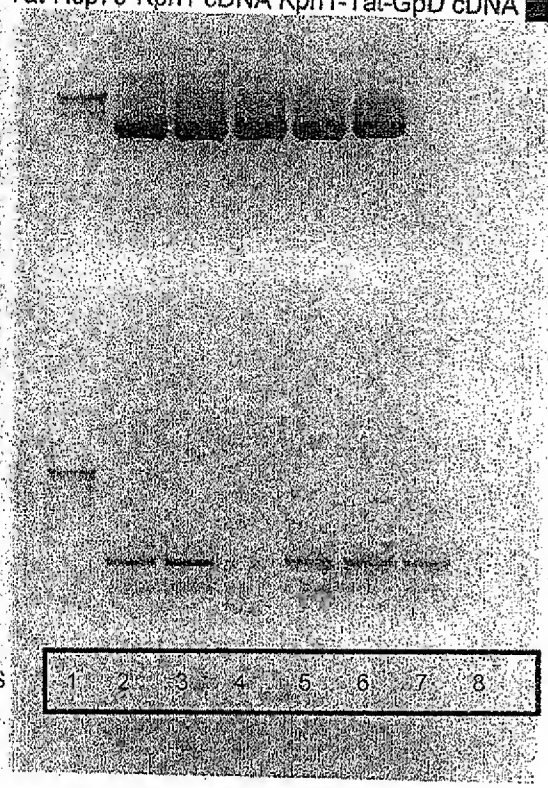
10. I declare that all statements made herein of my own knowledge and belief are true and that all statements made on information and belief are believed to be true, and further, that the statements are made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 04/04/2011

Shaharyar Khan

EXHIBIT 1

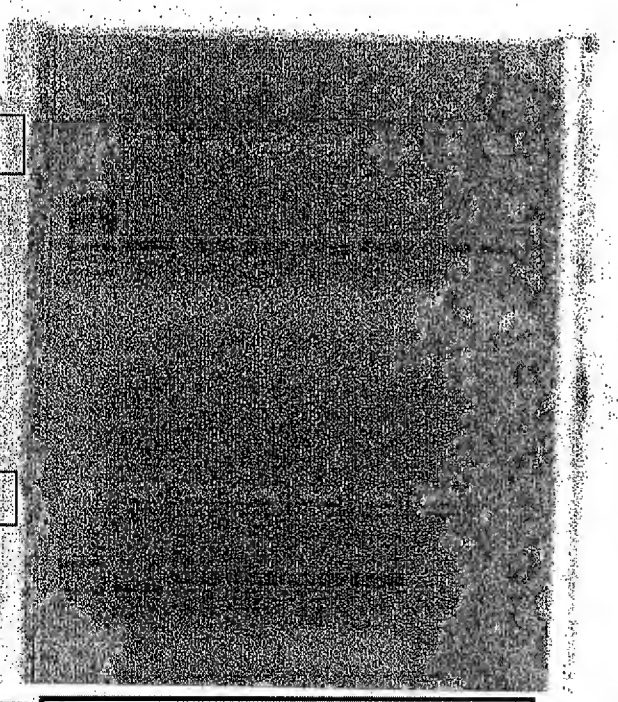
Tat-Hsp70-Kpn1 cDNA Kpn1-Tat-GpD cDNA [redacted]



1A

LANES 1 2 3 4 5 6 7 8

KpnI-gpD-BglII KpnI-Tat-gpD-BglII BglII-mtGFP-NotI [redacted]



1B

1C

LANES 1 2 3 4 5 6 7 8

See next page for Sequencing Results